6. Comparisons between the absorptions of the proteins of human serum reveal absorption bands for the horse somewhat greater in dimensions than those for the human.

7. The optical properties of the proteins of serum have been investigated with fairly satisfactory results.

8. Processes for the separation and purification of the proteins have been elaborated.

# *The Colouring Matter of Red Roses.*

By Geoffrey Currey.

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An examination of the petals of the red rose "George Dickson," has shown that the anthocyan pigment contained therein is the cyanidin glucoside, cyanin. It is present to the extent of about 9–10 per cent. by weight of the dried petals, and exists in the petals as an oxonium salt (*i.e.*, in combination with a plant acid). A yellow glucoside sap-pigment also occurs in the same flowers, but beyond the fact that it has been shown to be capable of producing an anthocyan, by reduction, and that it is not a glucoside of the flavonol myricetin, it has not been further identified, on account of the small quantity present. Further work may show it to be a glucoside of quercetin, and corroborate the work of Dr. Everest,* on the purple-black viola, in which it was shown that an anthocyan ("violanin") and the flavonol glucoside from which it could be produced, by reduction (a glucoside of myricetin), are present, side by side, in the same flowers. This would be additional evidence in favour of the hypothesis that "anthocyans are produced, in nature, by the reduction of the flavonols." It is interesting to note that this rose, grown in Australia, contains the same colouring matter as was isolated by Willstätter and Nolan† from the rose known as "Rosa Gallica," grown in Europe, and shows how widely these colouring matters are distributed in nature.

The rose "George Dickson" was chosen for this investigation on account of its deep red colour, which would indicate a fairly large percentage of the anthocyan pigment. The flowers from which the petals were gathered were grown by Mr. G. Knight, at his nursery, Parramatta Road, Homebush, and his generosity in supplying me with sufficient material enabled the work to be successfully accomplished.

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* 'Roy. Soc. Proc.,' B, vol. 90, p. 255 (1918).

† 'Annalen,' vol. 408, p. 1 (1915).

For the isolation of the anthocyan pigment, the methods used by Willstätter and Nolan, in their investigation of the "Rosa Gallica," were adopted, while the examination of the flavonol pigment was carried out on somewhat similar lines to that used by Dr. Everest in his examination of the viola.

## EXPERIMENTAL.

### *Isolation of the Anthocyan Pigment.*

100 grm. of the petals (which had been first air-dried in the shade, at room temperature, and finally over concentrated sulphuric acid), were allowed to stand in a closed vessel, with about 300 c.c. of methyl alcohol, containing 2 per cent. of concentrated hydrochloric acid (to prevent pseudo-base formation), for about twenty-four hours; the mass was then pressed, to obtain as much extract as possible, and the residue treated with a further quantity of methyl alcoholic hydrochloric acid. After standing some hours, this was filtered, with suction, and as the extraction was still incomplete, the residue was again extracted with the same solvent. The residue after filtering and washing possessed but a pale pink colour, and further extraction was deemed unnecessary. The filtrates and washings were united; they possessed a fine, deep red colour, with a bluish-violet tinge at the edge of the solution. The combined filtrates and washings were poured into about three times their volume of ether, well agitated, and allowed to stand for some hours. Practically all the anthocyan pigment separated out as a dark brown, gummy mass, from which the supernatant solution could be readily decanted.

The crude anthocyan pigment was redissolved in methyl alcoholic hydrochloric acid, and reprecipitated with ether (about two and a half times the volume of the alcoholic solution). After standing some hours, to allow precipitation to be as complete as possible, the ether-alcohol solution was decanted off and the precipitate allowed to stand for twenty-four hours in contact with a mixture of methyl alcohol and glacial acetic acid (to remove impurities capable of being hydrolysed or acetylated), and finally collected on a filter, washed with a small quantity of methyl alcohol (containing 1 per cent. hydrochloric acid), and air-dried.

The dark brown powder thus obtained was dissolved in boiling water, an equal volume of ethyl alcohol (containing 3 per cent. hydrochloric acid) added, and the solution allowed to cool; the colouring matter separated out in the form of dark brown leaflets, possessing a golden reflex. These were collected and air-dried. For identification purposes the anthocyanin chloride thus obtained was examined for the following properties, viz., crystalline form, colour, and reflex; colour changes on the addition of ferric chloride to aqueous and alcoholic solutions; colour of solutions in aqueous acid and alcohol; colour changes with alkalies; behaviour with Fehling's solution (hot and cold); colour of precipitate with lead acetate; behaviour with sodium bisulphite; behaviour with zinc and dilute acid; solubility in ethyl alcohol at 19° C.; solubility in aqueous hydrochloric acid (1 per cent. and 1·5 per cent.) at 20° C.; and the distribution of the pigment between amyl alcohol and dilute aqueous acid.

On comparing these properties with those given by Willstätter and Nolan for cyanin chloride, they were found to be identical; the anthocyan pigment of the red rose "George Dickson" is, therefore, the di-glucoside cyanin.

### *Hydrolysis of the Cyanin Chloride.*

The hydrolysis was carried out by boiling a small quantity of the glucoside pigment with 20 per cent. hydrochloric acid for three minutes; on standing, the sugar-free pigment separated in small needles (not long ones, as obtained by Willstätter), possessing a metallic lustre. On examination these were found to possess the same properties as described by Willstätter and Everest for cyanin chloride.

### *Examination of the Yellow Sap-Pigment.*

The ether-alcohol liquor, decanted from the anthocyan precipitate, was shaken with powdered calcium carbonate, and, after being allowed to stand for a short time, filtered. The filtrate, which possessed a clear, deep yellow colour, was concentrated to a small bulk and treated in the following manner:—A portion was evaporated to dryness and the residue extracted with ether; the ether solution was washed with dilute hydrochloric acid, to free it from traces of anthocyan pigment, filtered, and the filtrate shaken with dilute sodium carbonate solution, which became yellow in colour. Having shown that this alkaline solution contained the pigment in the form of a glucoside, the remainder of the concentrate was poured into water and boiled, to expel the small quantity of alcohol still present; the aqueous solution was then boiled with hydrochloric acid, to hydrolyse the glucoside present, allowed to cool, and extracted with ether. The ether extract was washed several times with aqueous acid, of different strengths, to ensure the removal of traces of anthocyanidin, and finally filtered. The filtrate was then shaken with dilute alkali, when the pigment passed into the alkaline layer, which assumed a deep yellow colour (the absence of a green colour here shows the absence of myricetin), which rapidly became dark brown on exposure to air (a possible explanation of this oxidation is given later). The alkaline solution was acidified, extracted with ether, and the ether extract evaporated to dryness. The residue, on being dissolved in absolute alcohol, to which a small quantity of hydrochloric acid had been added, yielded, on the addition of a small piece of magnesium ribbon, a fine red coloration, similar to an alcoholic solution of cyanidin chloride. It is possible that the yellow pigment present in the petals is a quercetin glucoside, but it was not possible to make further tests, as the quantity of pigment available was very small. In order to add additional weight to the assumption that a glucoside of quercetin is present, the above-mentioned tests were carried out with a known glucoside of quercetin, viz., "myrticolorin." A small quantity was boiled with acid and hydrolysed, and the resulting sugar-free pigment "quercetin" treated, in alcoholic solution, with hydrochloric acid and magnesium ribbon in the same manner as with the sugar-free pigment from the rose, exactly similar results were obtained. Tests made with both pigments in a glucoside form also agreed. It is hoped that further work on this rose will result in sufficient of the yellow flavonol sap-pigment being isolated to confirm its identity, by means of derivatives. For the sample of "myrticolorin" used in the above tests, I am indebted to Mr. H. G. Smith, of the Technological Museum, who very kindly supplied me with it; it is a rhammo-glucoside of quercetin, and was isolated from eucalyptus leaves.

### *Possible Cause of the Rapid Darkening of the Alkaline Solution of the Yellow Sap-pigment from the Rose.*

Although it is the property of the flavonols to undergo oxidation on exposure to air in alkaline solution, the very rapid oxygen absorption of the solution previously mentioned would appear to be due to some other cause. It was thought that this might possibly be due to tannin matter. An examination of the petals of the rose revealed the fact that both pyrogallol and catechol tannins were present, the former (probably gallo-tannin), being present in the greater quantity. The alcoholic extract of the petals would contain the tannin matter in solution, and the boiling with acid, to hydrolyse the glucosides, as previously mentioned, would also hydrolyse the gallo-tannin, with the production of gallic acid; the subsequent extraction of the acid solution with ether, to obtain the hydrolysed glucosides, would also extract the gallic acid, and this would accompany them into the alkaline solution; the presence of a small quantity of gallic acid would be quite sufficient to cause a rapid darkening of the solution, on exposure to air.